45th ANNUAL ICAAC Washington, D.C. December 16-19, 2005

M-1610

Comparison of the Sensititre® YeastOne and Fungitest® methods with the NCCLS M27-A2 reference method for antifungal susceptibility testing of yeasts.

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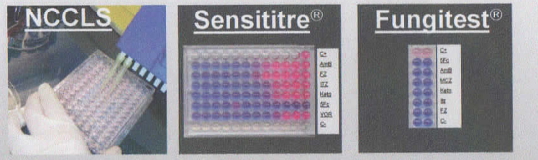
ABSTRACT

Background. The recent introduction of the Sensititre® YeastOne (Trek diagnostic), a colorimetric microdilution method that includes new antifungal agents opens the field to MICs determination by an easy-to-perform method. The aim of this study was to compare this test with the reference NCCLS M27-A protocol and with Fungitest® (Biorad), a current routine method for yeasts susceptibility testing. **Methods.** Sensititre® YeastOne and the NCCLS M-A2 methods were performed on 300 clinical isolates of yeasts including 125 *C. albicans*, 175 C. non-*albicans* species. Two ATCC strains were included as controls. Four antifungal agents were tested by the reference method: amphotericine B (AmB), fluconazole (FZ), itraconazole (ITZ) and voriconazole (VOR). The reading of the Sensititre® and NCCLS results was visually performed at 24 and 48 h respectively. The Fungitest® method (including AmB, FZ and ITZ) was applied to 121 among the 300 isolates and the reading was done between 24 and 48 h of incubation according to the growth of the positive control. **Results.** By the NCCLS method the MIC_{50}/MIC_{90} (µg/ml) were as follow: 1/2 (AmB); 16/64 (FZ); 0.25/4 (IT) and 0.125/2 (VOR). Sensititre® vs. NCCLS. The overall agreements within 2 dilutions for AmB, FZ, ITZ and VOR were respectively 54, 82, 80 and 78%. Very major errors (%) were recorded: 0.01/0 (AmB with a MIC ≥ 4/8µg/ml for resistant strains respectively), 3.6 (ITZ), 1.6 (FZ) and 2.3 (VOR with a MIC ≥ 8µg/ml for resistant strains). Fungitest® vs NCCLS. The agreement between both methods including minor discrepancies was 98% (AmB), 88% (FZ) and 98% (ITZ). Following the breakpoints given by the manufacturer, very major errors were 6.3% for FZ, 0.03% for ITZ and none for AmB. **Conclusions**. Sensititre® is a convenient alternative to the NCCLS method for yeast susceptibility testing. For Fungitest®, in spite of good correlations, the breakpoints should be changed and to be competitive, new antifungal agents should be included.

Introduction

Significant progress concerning the development of standardized testing methods for antifungal agents has been achieved through the NCCLS. However the NCCLS reference method is time consuming and labor intensive and is difficult to introduce as a routine technique. Commercial testing systems based on microdilution method are know available. Fungitest® allows the testing of 6 antifungal agents at two different concentrations: amphotericine B (AmB), 5 fluorocytosine (5FU), miconazole (MZ), ketoconazole (KZ), fluconazole (FZ) and itraconazole (IZ). Sensititre® YeastOne, is a microdilution method including 11 dilutions of 7 antifungal agents including new agents like voriconazole and caspofungin. The aim of this study was to compare the two commercial methods with the reference NCCLS M27-A2 protocol.



Materials and Methods

Clinical isolates: 125 *C. albicans*, 175 *C. non albicans* collected at the University Hospital of Liège in 2003-2004. The distribution of the *non albicans* isolates was: 85 *C. glabrata*, 55 *C. parapsilosis*, 12 *C. tropicalis*, 6 *C. krusei*, 5 *C. lusitaniae*, 2 *C. guillermondii*, 1 *Geotrichum capitatum*, 1 *C. sake*, 5 *S. cerevisiae*, 3 *Cryptococcus neoformans*, The identification has been performed either by Bichrolatex ® (Fumouze, France) or by Api 32C panel (Biomérieux , France).

Control strains: *C. albicans* ATCC10231, *C. glabrata* ATCC 90030 (ISP, Bruxelles).

Methods: Sensititre® YeastOne (Trek diagnostic systems, UK) and **NCCLS M27-A2** method were performed on 300 clinical isolates. Four antifungals were tested by the NCCLS method: AmB, ITZ, VOR and FZ. Six agents were tested in the Sensititre® panels: amB, ITZ, VOR, FZ, 5 Fc, and KZ.. The reading of Sensititre® panels was performed after 24 hours incubation time at 37°C with the aid of a reading mirror. For NCCLS method the reading was performed after 48 hours incubation at 37°C, visually and spectrophotometrically at 405 nm. Fungitest® (Biorad, France) was performed on 121 among the 300 clinical isolates. The results were reported visually after 24 to 48 hour incubation according to the growth of the positive control. N.B. This work was performed with support of Trek® laboratories.

Results

1. MIC_{50} and MIC_{90}.

Table 1. $MICs_{50}$ and MIC_{90} given by the NCCLS method.

Antifungal agents	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
Amphotericin B	1	2
Fluconazole	16	64
Itraconazole	0.25	4
Voriconazole	0.125	2

2. Overall agreement between MIC pairs.

Table 2: Percentage of agreement between Sensititre and Fungitest vs. NCCLS method (±2 dilutions)

Antifungal agents	Sensititre (%)	Fungitest (%)
Amphotericine B	54	98
Fluconazole	82	88
Itraconazole	80	98
Voriconazole	78	Not tested

3. Very major errors.

Table 3. Sensititre® and Fungitest® vs. NCCLS.

Very major errors (%)	Sensititre	Fungitest
Amphotericin B	0,01*/0**	0
Fluconazole	1,6	6,3
Itraconazole	3,6	0,03
Voriconazole	2,3°	Not tested

*Resistant strains for MIC ≥4 µg/ml;
**Resistant strains for MIC ≥8 µg/ml
°Resistant strains for MIC ≥ 8 µg/ml

Results

1. Resistant isolates.

Table 4. Percentage of resistant isolates for the most representative *Candida* species.

MIC (µg/ml) / (*Candida* species)	AmB MIC ≥4 (%)	ITZ MIC ≥ 4 (%)	FZ MIC ≥ 64 (%)	VOR MIC ≥ 8 (%)
C. albicans (n=125)	0	10,4	5,6	8
C. glabrata (n=85)	0	17,6	14,6	9,4
C. parapsilosis (n=55)	2	2	41,8	3,6
C. tropicalis (n=12)	0	16,7	0	16,7

Discussion

- Both commercial methods are simple to perform and the colorimetric reading is less interpretative that those recommended by the reference method.
- Sensititre is more flexible: the panel includes a new azole, voriconazole (and more recently caspofungin, not yet available at the time of the study). Both agents are lacking in the Fungitest panel. Furthermore, the use of only two concentrations in Fungitest does not allow MIC's determination.
- In this study the $MICs_{50}$ and MIC_{90} are surprisingly high with fluconazole, possiblly depending of the selected isolates.
- The percentage of agreement between both method versus NCCLS is quite good. However, for amphotericin B, Sensititre gives a very low percentage of agreement. Very major discrepancies were reported for both methods mostly with azoles. The higher discrepancy concerns fluconazole by Fungitest method as reported by other author's.

Conclusion.

Sensititre is a convenient alternative to the NCCLS method for yeast susceptibility testing. For Fungitest, in spite of good correlations, the breakpoints should be changed, and to be competitive, new agents should be included.